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Review

Plant sterols, cholesterol precursors and oxysterols: Minute concentrations—Major physiological effects

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ABSTRACT

Non-cholesterol sterols are present in our body at very low concentrations as compared to cholesterol. Small changes in the structure of sterol molecules confer them highly distinct biological activities. The best-known example are steroid hormones derived from cholesterol. During the past decade, our knowledge of also other biomolecules related to or derived from cholesterol, particularly plant sterols, biosynthetic precursors of cholesterol, and oxysterols, has expanded rapidly. In this review article we recapitulate the latest insights into the properties and physiological activities of these non-cholesterol sterols, as well as their importance in disease processes and potential as diagnostic biomarkers.

Contents

1. Introduction

Cholesterol is a lipid essential for all mammalian cells, present at high concentrations in cellular plasma membranes and certain intracellular organelles, as well as in plasma lipoproteins. It is by far the most abundant individual lipid molecular species within our body [1,2]. We obtain cholesterol from two sources, from nutrition and as synthesised *de novo* by our cells, particularly hepatocytes (Fig. 1). Cholesterol in the nutrition is absorbed by enterocytes, which transfer it into the circulation *via* mesenteric lymph, after which it is taken up by the liver as a constituent of chylomicron remnants. The liver packages the nutritional and the endogenously synthesised cholesterol into lipoproteins (VLDL and HDL) that it secretes into the bloodstream. The liver is also the major organ taking up lipoprotein (VLDL, LDL, HDL) cholesterol and excretes it into bile ducts as such or after conversion into bile acids (Figs. 1 and 2).

Cholesterol concentrations in human serum are in the range of millimoles/liter (mM), but there are also other, so-called non-cholesterol sterols found in the circulation and in tissues at

Abbreviations: ABC, ATP-binding cassette (transporter); HDL, high-density lipoproteins; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LDL, low-density lipoproteins; LXR, liver X receptor; NPC1L1, Niemann-Pick C1-like 1; VLDL, very-low-density lipoproteins.

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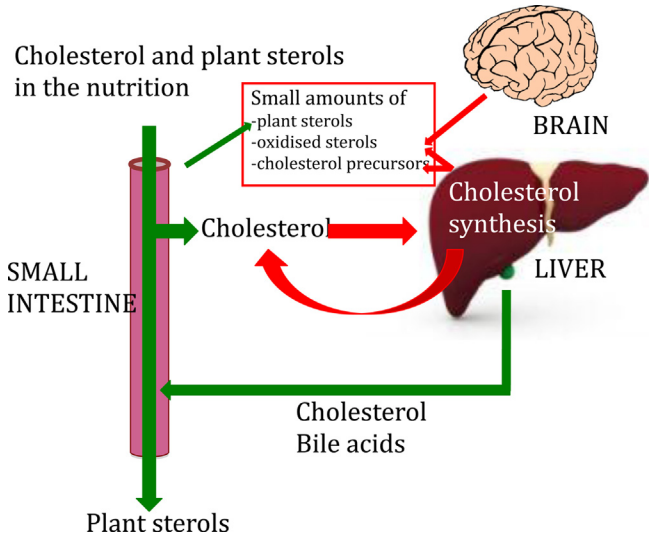

Fig. 1. Overview of the transport of cholesterol and non-cholesterol sterols in the human body. The red arrows depict sterol transport in the blood/lymph and the green ones in the intestine or bile ducts. Under normal circumstances dietary cholesterol is efficiently absorbed, while the vast majority of plant sterols are returned into the intestinal lumen. Non-cholesterol sterols appear in the circulation and tissues at very low concentrations. Plant sterols originate from the diet, cholesterol precursors mainly from the liver, and oxysterols from the brain, lungs, liver and other tissues (not shown). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

markedly lower, nM or at the most μM concentrations. The most abundant of these are plant sterols and stanols originating from plant-derived foodstuffs, biosynthetic precursors of cholesterol, and oxidised derivatives of cholesterol, oxysterols. A vast majority of plant sterols in the nutrition remain unabsorbed and are removed from the body in feces; However, small amounts of these compounds enter the system (Fig. 1). Also cholesterol precursors enter into the circulation at low concentrations from tissues, mainly from the liver. The same holds true for oxysterols, which are generated in small amounts in many tissues, the most important of which are the liver, the lungs and the brain (Fig. 1).

Cholesterol precursors and oxysterols are more hydrophilic than cholesterol. This is why their mobility in cells and capability of penetrating membranes are markedly higher than of cholesterol, and their half-life in the system much shorter [3]. The concentrations of these molecules therefore vary in a more dynamic manner than cholesterol, and they can be employed as sensitive indicators of cholesterol metabolism. In this review we present an overview of the latest insights into the mechanisms of action of plant sterols, cholesterol precursors, and oxysterols within out body, their significance in disease processes, and their potential as diagnostic biomarkers.

1.1. The entry of plant sterols and stanols into circulation is efficiently precluded

The western-type diet contains an average of approximately 300 mg cholesterol per day. In addition, it contains roughly the same amount of plant sterols (approximately 300 mg/day) and stanols with a saturated cyclical structure (approximately 50 mg/day).

The Niemann-Pick C1-like protein 1 (NPC1L1) responsible for cholesterol absorption in enterocytes also mediates the uptake of plant sterols and stanols by the intestinal epithelium, but most likely at a lower efficiency than cholesterol [4]. The ABCG5 and ABCG8 transporters form a membrane-associated pump that consumes the energy of ATP to remove plant sterols and stanols from enterocytes back into the intestinal lumen, so that only 0.5–2% of plant sterols and 0.04–0.2% of stanols enter the system [5]. ABCG5 and −8 also function at the hepatocyte plasma membrane domain facing bile ducts, removing the plant sterols that have entered hepatocytes, into bile and further on to the intestine [6]. Moreover, these ABC transporters pump cholesterol from hepatocytes to bile ducts (Fig. 2). Serum plant sterols can be employed as biomarkers for the rate of intestinal cholesterol absorption and for diagnostics of sitosterolemia, also denoted as phytosterolemia.

Sitosterolemia is a rare autosomal recessively inherited disease, in which mutations in the ABCG5 or −8 transporters result in strongly elevated concentrations of plant sterols and stanols in serum and tissues [4]. Additionally, this condition involves almost exclusively hypercholesterolemia due to the function of these transporter proteins in the routing of cholesterol from hepatocytes to bile ducts (Fig. 2). In sitosterolemia, the serum concentrations of plant sterols, the most abundant of which are sitosterol and campesterol, are typically 0.1–1 mM, i.e. approximately 50-fold higher than the normal levels. This inherited disorder of lipid metabolism results in the formation of skin and tendon xanthomas, structural alterations in erythro- and thrombocytes, hemolysis, and in some patients in an increased cardiovascular

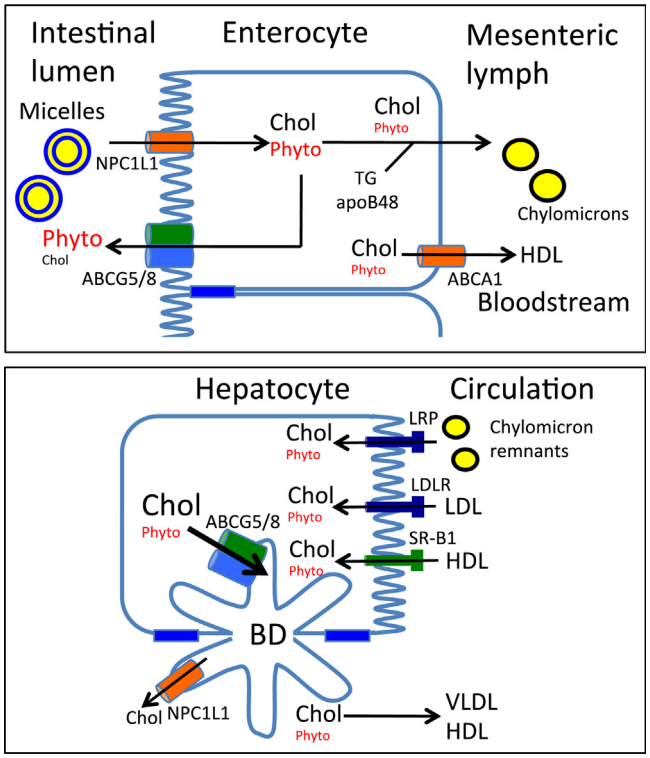

Fig. 2. Overview of the transport of cholesterol (Chol) and plant sterols (Phyto) in enterocytes and hepatocytes. Cholesterol and part of the plant sterols in the nutrition are absorbed into enterocytes *via* the NPC1L1 transporter, but an efficient retrograde transport mechanism driven by ABCG5/8 recycles a vast majority of the plant sterols into the intestinal lumen. Sterols are secreted from enterocytes into lymph as incorporated in chylomicrons and into bloodstream in HDL, and are taken up by the liver *via* lipoprotein receptors. The liver also synthesises cholesterol. Sterols are partly excreted into bile ducts and partly packaged into VLDL and HDL particles for secretion into the circulation. VLDL in the circulation is converted *via* hydrolysis of constituent lipids into smaller LDL particles, the majority of which are reinternalized into the liver *via* LDL receptors. Likewise, the majority of the HDL produced by the liver and the small intestine returns to the liver. NPC1L1, Niemann-Pick C1-like protein 1; ABC, ATP-binding cassette transporter; apoB48, apolipoprotein-B48; TG, triglyceride; BD, bile duct; LDLR, LDL receptor; LRP, LDL receptor-related protein; SR-B1, scavenger receptor class B, type 1.

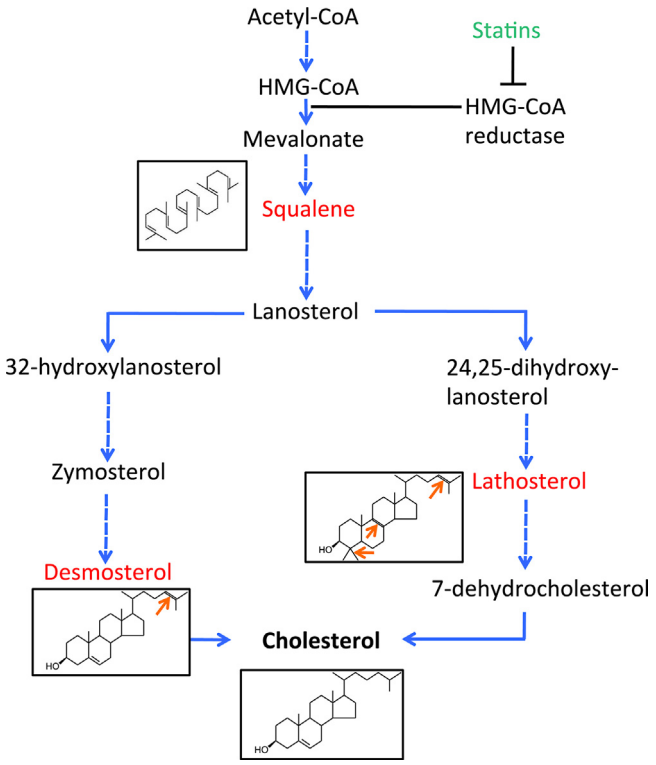

Fig. 3. Simplified scheme of cholesterol biosynthesis. The precursors mentioned in the text are indicated with red, and their structures are depicted. The orange arrows point to differences in structure as compared to cholesterol. Statins inhibit the enzyme HMGCoA-reductase in the early part of the biosynthetic pathway. CoA, coenzyme A; HMG, 3-hydroxy-3-methylglutaryl.approximately 1/1000 of that of cholesterol. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

risk. One should suspect sitosterolemia in a patient associated with any of the above clinical observations (even if serum cholesterol concentrations are within the normal range). The diagnosis can be verified by gas chromatography analysis of serum sterol profile and investigation of ABCG5 and −8 gene defects [7].

Ever since the first sitosterolemia patients were reported in the 1970s, researchers have been wondering whether increased cardiovascular risk is associated with elevated serum concentrations of plant sterols. Some of the patients do present with early onset atherosclerosis, but for instance in a recent French study on five sitosterolemia patients at ages 11–32 years, none of the subjects were found to suffer from atherosclerotic arterial disease [8]. The putative association between plant sterols and atherogenesis has also been investigated in the normal population. The results from a number of cross-sectional or prospective studies, meta-analyses and gene polymorphism studies have been controversial, ranging from protective effects of plant sterols to harmful, pro-atherogenic roles of these compounds [9–14]. Since the serum plant sterol concentrations in the normal population are suggested by several laboratories to act as biomarkers for cholesterol absorption [15], it is possible that in those studies in which plant sterol concentrations correlate with the prevalence of arterial disease, the actual risk factor is a high efficiency of cholesterol absorption and not the plant sterol itself. To conclude, a possible role of plant sterols in the development of atherosclerotic arterial disease is as yet poorly understood. Thus, further research on this topic is warranted.

1.2. Cholesterol precursors reflect activity of the sterol biosynthetic pathway

The cellular cholesterol synthesis takes place *via* a complicated chain of reactions involving more than 30 enzymes (Fig. 3). The synthesis begins at acetyl-coenzyme A and leads through isoprenoids to cyclical precursors that are further modified to the final product, cholesterol [16]. A rate-limiting enzyme in the pathway, 3-hydroxyl-3-methylglutaryl coenzyme A, is the target inhibited by statins (Fig. 3). Squalene mono-oxygenase, which oxidises squalene to form the first cyclical intermediate in the pathway, lanosterol, is another enzyme limiting the rate of cholesterol biosynthesis [17]. Cholesterol precursors (most commonly squalene, lathosterol, and desmosterol; Fig. 3) leak from tissues into the circulation in amounts that correlate positively with activity of the synthetic pathway. This is why the ratio of the precursor molecules to cholesterol in serum is used as an indicator of the activity of the sterol biosynthetic pathway. The concentrations of the above three precursors in serum are on the average (and range) as follows in population based studies: squalene 1.7 (1.2–2.1) μmol/l, lathosterol 10 (2–20) μmol/l, and desmosterol 5 (2–7) μmol/l, i.e. approximately 1/1000 of that of cholesterol [18–20].

In addition to their roles as biomarkers for cholesterol synthesis, individual cholesterol precursors have further useful properties. E.g., serum desmosterol concentration was reported to be higher in patients with non-alcoholic steatohepatitis than in subjects with mere hepatic steatosis [21]. Moreover, measuring the concentrations of 7- and 8-dehydrocholesterol is of use when suspecting the congenital Smith-Lemli-Opitz syndrome (SLOS). SLOS caused by mutations in 7-dehydrocholesterol reductase is characterised by a deficiency of cholesterol, high concentrations of 7- and 8-dehydrocholesterol, and developmental anomalies.

The brain is the most cholesterol-rich organ and produces almost all of its cholesterol endogenously. Desmosterol reaches very high concentrations (up to 30% of total sterols) during the development of mammalian brain, suggesting an important and specific functional role of this precursor sterol in the development of the central nervous system [22]. The fact that mother's milk contains a lot of desmosterol may also bear relevance in terms of this hypothesis [23]. The mechanisms underlying the accumulation of desmosterol in the developing brain are not fully understood. We have suggested that one contributing factor could be the high level of progesterone during gestation; Progesterone inhibits post-transcriptionally the enzyme that converts desmosterol into cholesterol [22].

Alterations in cholesterol precursors potentially associated with pathogenesis have been observed in neurodegenerative disorders, such as Alzheimer's disease. We observed that serum concentrations of squalene and lathosterol are significantly reduced in familial Alzheimer's disease, which may reflect a reduction of hepatic cholesterol biosynthesis [24]. Whether this also reflects decreased production of cholesterol within the Alzheimer's disease brain is thus far unclear. In some studies the use of statins has been reported to act protective of Alzheimer's disease. This may, however, associate with other biological activities of statins than cholesterol lowering [25].

1.3. Oxysterols—regulators of sterol metabolism and biomarkers of oxidative stress

Oxysterols are oxidised derivatives of cholesterol or by-products of its biosynthesis [26]. The most abundant oxysterols arise intracellularly as products of enzymatic cholesterol oxidation,

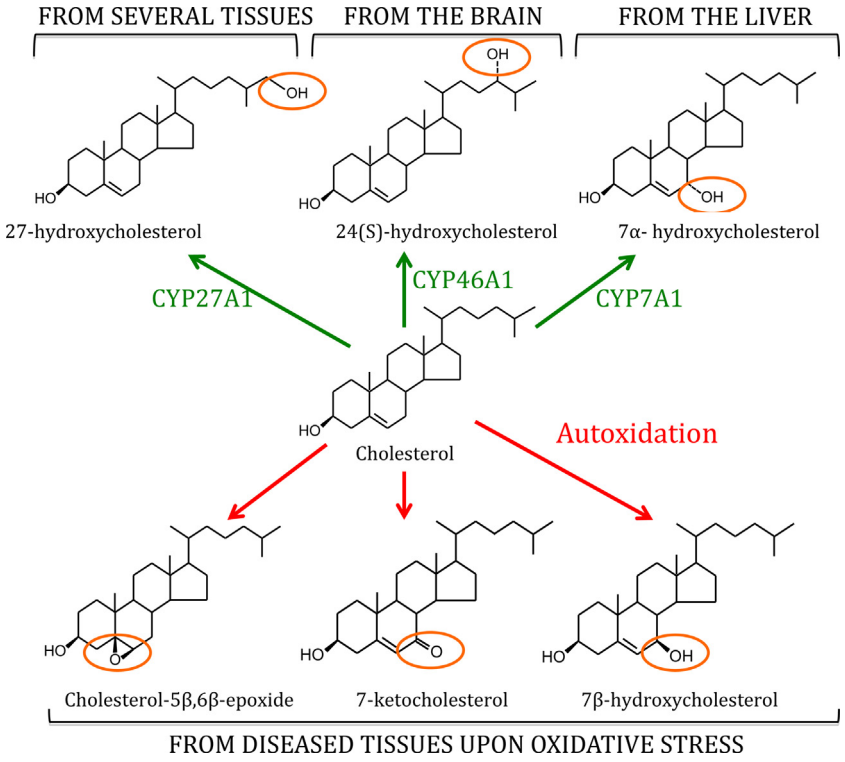

Fig. 4. Generation of some abundant oxysterols from cholesterol. The species in the top row arise through the action of cholesterol hydroxylases belonging to the cytochrome P450 family (green). The species at the bottom arise through non-enzymatic oxidation (autoxidation) of cholesterol. Structural differences as compared to cholesterol are indicated in orange. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

the other major mechanism of oxysterol generation being non-enzymatic oxidation of cholesterol, termed autoxidation.

(Fig. 4). Cholesterol autoxidation products arise upon the processing of foodstuffs but also within our body *in vivo*. Oxysterol concentrations in the serum of healthy subjects are extremely low, the most abundant species reaching maximally 100 nM (<1/10000 of the concentration of cholesterol). Of the enzymatically arising oxysterols, 27-hydroxycholesterol originates from the lungs [27] and several other tissues while 24(S)-hydroxycholesterol in mainly generated in the brain [28,29](Fig. 4). Neurons extrude cholesterol by converting it to 24(S)-hydroxycholesterol, also denoted as cerebrosterol, which can be used as a biomarker for cholesterol egress from the brain [30]. The enzymatic oxysterols have a number of beneficial biological effects, the most important of which are mediated by the liver X receptor (LXR) transcription factors [31]. Activation of LXRs by oxysterols enhances the removal of cholesterol from macrophage foam cells to HDL, from enterocytes to the intestinal lumen and to HDL particles secreted by these epithelial cells [32], as well as dampens the expression of pro-inflammatory cytokines and protects cells from apoptosis [31]. Physiological concentrations of enzymatic oxysterols are thus, according to the current view, considered to act as anti-atherogenic compounds.

Concentrations of oxysterols in serum may rise under pathological conditions involving oxidative stress, such as familial combined hyperlipidemia, atherosclerosis, obesity, insulin resistance, and advanced kidney disease [33,34]. The oxysterols enriched in these disease states mainly represent products of non-enzymatic cholesterol oxidation, such as 7β-hydroxycholesterol and 7-ketocholesterol (Fig. 4). These oxysterol species can be considered as markers of oxidative stress. They are also enriched in pathological tissues, of which atherosclerotic lesions and macrophage foam cells are most extensively studied [35]. The oxysterols accumulating in atherosclerotic plaques are known to enhance inflammatory reactions and to cause cell death, which is believed to promote formation of the necrotic plaque core and destabilization of plaques [35,36]. Of note, elevated concentrations of 7-ketocholesterol and cholestane-3β,5α,6β-triol are found in various tissues and in blood plasma of patients with Niemann–Pick type C disease [37–40]. Gene defects in cholesterol 27-hydroxylase (CYP27A1) lead to reduced bile acid synthesis with a compensatory increase in the activity of the rate-limiting enzyme in bile acid synthesis, cholesterol 7α-hydroxylase. The resulting disease termed cerebrotendinous xanthomatosis (CTX) is characterized by low levels plasma 27-hydroxycholesterol and accumulation of 7α-hydroxylated bile acid precursors, in particular 7α-hydroxy-4-cholesten-3-one and its derivative cholestanol [41] as well as certain oxysterol species [42,43]. Plasma oxysterols can thus be employed for the diagnosis of the above rare inherited diseases.

2. Conclusions and future perspectives

Non-cholesterol sterols are detected in our body at very low concentrations. They can be employed as biomarkers for both of the major routes through which the body acquires cholesterol, absorption from the nutrition and endogenous biosynthesis: Plant sterols, such as sitosterol and campesterol, are employed as biomarkers for sterol absorption, and cholesterol precursors, such as lathosterol and desmosterol, as markers of cholesterol synthesis. Oxysterol species arising *via* non-enzymatic cholesterol oxidation reflect oxidative stress. In addition, oxysterols arising through enzymatic oxidation of cholesterol can be employed to estimate how efficiently cholesterol is removed from certain tissues, such as the brain. However, it is as yet unclear to what extent this information is useful in designing therapy: e.g., the choice of statin (inhibition of cholesterol synthesis and enhancement of its hepatic absorption) *vs*. ezetimibe therapy (inhibition of cholesterol uptake

via the intestine and elevation of its synthesis), or a combination thereof. Descamps et al. [44] recently reviewed the eight major human trials where cholesterol absorption and synthesis were analyzed on a large scale using the plasma levels of precursors of cholesterol and plant sterols. At baseline, the inverse relationship between cholesterol absorption and synthesis (only examined in two studies) was found to be weak. On statin treatment, four studies showed that the changes in cholesterol synthesis and absorption contributed less than 9% to the variability in cholesterol response to statin therapy. Moreover, it has not been consistently demonstrated that good absorbers/bad synthesizers are bad responders to statin and good responders for ezetimibe. The authors concluded that, with the exception of a reverse effect of statin and ezetimibe on cholesterol absorption and synthesis, most ideas supporting the interplay between these two processes lacked consistency between the published studies. Thus, the use of the plasma levels of plant sterols and cholesterol precursors as markers of cholesterol absorption and synthesis is at present too limited to definitively evaluate their value in clinical practice. Large studies addressing the relationship of cholesterol absorption and synthesis, their optimal measures, and their usefulness in patient care are thus warranted and necessary. We believe that wide spectrum profiling of sterols can in the future provide detailed knowledge of a subject's sterol metabolism and help in the diagnosis and design of optimal treatment.

Disclosure

The authors declare no conflict of interest concerning the contents of this article.

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